UNIVERSITI PUTRA MALAYSIA

COMPARATIVE STUDIES ON GANODERMA (KARST.) FROM INFECTED OIL PALM AND COCONUT STUMPS WITH SPECIAL REFERENCE TO THEIR MORPHOLOGICAL, MOLECULAR AND ISOZYME CHARACTERISTICS

LATIFFAH ZAKARIA

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By

LATIFFAH ZAKARIA

Thesis Submitted in Fulfilment of the Requirement for the Degree of
Doctor of Philosophy in the Institute of Bioscience
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Abstract of the thesis submitted to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the Degree of Doctor of Philosophy

A COMPARATIVE STUDY ON *GANODERMA* (KARST.) FROM INFECTED OIL PALM AND COCONUT STUMPS WITH SPECIAL REFERENCE TO THEIR MORPHOLOGICAL, MOLECULAR AND ISOZYME CHARACTERISTICS

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January 2002

Chairman: Prof. Dr. Ho Yin Wan

Faculty: Institute of Bioscience

The basal stem rot of oil palm caused by *Ganoderma* is the most serious disease infecting oil palm in South-east Asia. It is believed that coconut stumps which are colonized by *Ganoderma* can act as sources of inoculum for infection to healthy palms through root contact. However, it is not known whether the *Ganoderma* infecting oil palm and those colonizing coconut stumps are the same species. Therefore, a comparative study was conducted to determine the similarities and differences between *Ganoderma* isolates from infected oil palm and coconut stumps, using a multidisciplinary approaches in which morphological, biochemical (intracellular and extracellular enzyme systems) and molecular characteristics (RAPD, RAMS, RFLP and direct sequencing of the ITS regions + 5.8S gene of rDNA) were analysed.

Based on the morphological characteristics of the basidiomata, *Ganoderma* from infected oil palm and coconut stumps conformed to the description of *G. boninense* in Steyaert's classification system for *Ganoderma* (1967 and 1975). The growth on various media and at different temperatures, and the cultural characteristics of the isolates from infected oil palm and coconut stumps were similar with no significant difference observed between the two groups of *Ganoderma*. However, the isolates were somatically incompatible with one another, which indicated that they were genotypically distinct individuals and not clones of a genotypic individual.

The isozyme profiles from intracellular and extracellular enzyme systems, and the DNA profiles from RAPD, RAMS and RFLP of the ITS regions + 5.8S gene revealed that *Ganoderma* isolates from infected oil palm and coconut stumps were very variable. Nucleotide sequences of the ITS regions + 5.8S gene of rDNA from a limited number of isolates also showed that the isolates from both groups of *Ganoderma* were very variable. The Southern hybridization of RAMS gel showed that labelled probes from oil palm and coconut hybridized to the common bands of 1.2 kb by from primer $(CGA)_5$ and 1.4 kb band by primer $(ACA)_5$ which indicated that the bands of the same molecular sizes are likely to be homologous.

Cluster analysis based on data from biochemical and molecular characters, and phylogenetic analysis of the nucleotide sequence showed that the oil palm isolates and the coconut isolates did not cluster separately which indicated that isolates of both groups of *Ganoderma* are closely related.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

KAJIAN PERBANDINGAN *GANODERMA* (KARST.) DARIPADA KELAPA SAWIT DAN TUNGGUL KELAPA MENGGUNAKAN CIRI-CIRI MORFOLOGI, MOLEKUL DAN ISOZIM

Oleh

LATIFFAH ZAKARIA

Januari 2002

Pengerusi: Profesor Dr. Ho Yin Wan

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Penyakit reput pangkal batang yang disebabkan oleh kulat *Ganoderma* merupakan penyakit yang paling serius menjangkiti pokok-pokok kelapa sawit di Asia Tenggara. Penyakit ini dipercayai berjangkit daripada tunggul kelapa di mana ianya sebagai sumber inokulum, boleh menjangkiti pokok-pokok kelapa sawit yang sihat melalui persentuhan akar. Walaubagaimanapun, tidak diketahui samada *Ganoderma* yang menjangkiti kelapa sawit dan yang terdapat pada tunggul kelapa terdiri daripada spesis yang sama. Oleh itu, satu kajian perbandingan telah dijalankan untuk mengenalpasti persamaan dan perbezaan di antara isolat-isolat *Ganoderma* daripada kelapa sawit dan tunggul kelapa menggunakan pelbagai kaedah seperti ciri-ciri morfologi basidiomata, biokimia (menggunakan sistem enzim intrasel dan ekstrasel) dan molekul (menggunakan analisis RAPD, RAMS, RFLP dan jujukan 'ITS region + gen 5.8S').

Berdasarkan ciri-ciri morfologi basidiomata, didapati ciri-ciri *Ganoderma* daripada kelapa sawit dan tunggul kelapa menyerupai ciri-ciri *G. boninense* yang terdapat pada sistem klasifikasi *Ganoderma* oleh Steyaert (1967 dan 1975). Pertumbuhan miselium pada pelbagai media dan suhu, serta ciri-ciri kultur pertumbuhan isolat-isolat kelapa sawit dan kelapa menunjukkan persamaan dimana tiada perbezaan yang bererti di antara kedua-dua kumpulan *Ganoderma* tersebut.

Profil isozim daripada sistem enzim intrasel dan ekstrasel dan profil DNA daripada analisis RAPD, RAMS, RFLP dan jujukan 'ITS regions + gen 5.8S', menunjukkan terdapatnya variasi pada isolat-isolat kelapa sawit dan kelapa. Variasi juga dapat dilihat pada jujukan 'ITS regions + gen 5.8S' pada beberapa isolat kelapa sawit dan kelapa. 'Southern Hybridization' dari gel analisis RAMS menunjukkan probe daripada kelapa sawit dan kelapa menghibridasi kepada jalur-jalur yang mempunyai size molekul yang sama iaitu jalur 1.2 kb daripada primer $(CGA)_5$ dan jalur 1.4 kb daripada primer $(ACA)_5$, menunjukkan jalur yang mempunyai size molekul yang sama adalah homologus .

Analisis cluster daripada kaedah pencirian biokimia dan molekular, serta analisa filogenetik jujukan DNA menunjukkan isolat-isolat kelapa sawit dan kelapa tidak berkelompok secara berasingan. Oleh itu, daripada analisis cluster isolat-isolat *Ganoderma* daripada kelapa sawit dan kelapa mempunyai hubungan yang rapat.

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I certify that an Examination Committee met on 7th January 2002 to conduct the final examination of Latiffah bt. Zakaria on her Doctor of Philosophy thesis entitled "Comparative Studies on *Ganoderma* (Karts.) from Infected Oil Palm and Coconut Stumps with Special Reference to their Morphological, Molecular and Isozyme Characteristics" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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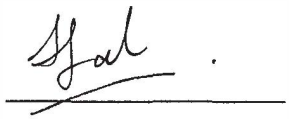
DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.



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Date : 30/1/2002

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